Pheromone Blends of Predaceous Bugs (Heteroptera: Pentatomidae: *Podisus* spp.)

J. R. Aldrich

USDA-ARS, Insect Chemical Ecology Laboratory, Agricultural Research Center-East, B-467, Beltsville, Maryland 20705, U.S.A.

J. P. Kochansky, W. R. Lusby

USDA-ARS, Insect Neurobiology and Hormone Laboratory, Agricultural Research Center-East, B-467, Beltsville, Maryland 20705, U.S.A.

and

M. Borges

EMBRAPA/CENARGEN, Centro Nacional de Recuros Geneticos e Biotechnologia, CEP 70.770 Brasilia DF, Brazil

Z. Naturforsch. **46c**, 264–269 (1991); received October 9, 1990

Hemiptera, Stink Bug, Attractant, (*E*)-2-Hexenal, α-Terpineol

Male predaceous stink bugs (Pentatomidae: Asopinae) in the genus *Podisus* release long-range attractant pheromones from a pair of hypertrophied glands opening underneath the wings. Pheromone compositions are reported for four additional *Podisus* spp.: two Neotropical species (*P. connexivus* and an undetermined *Podisus* sp.), and two Nearctic species (*P. placidus* and *P. mucronatus*). Males of each species release (*E*)-2-hexenal, plus species-specific major components that include α-terpineol, linalool, 9-hydroxy-2-nonanone, and (*E*)-2-hexenyl tiglate. The pheromonal chemistry of the Neotropical species closely resembles that for the previously studied Nearctic species, *P. maculiventris* and *P. fretus*.

Introduction

Stink bugs in the New World genus *Podisus* (Pentatomidae: Asopinae) are obligatory predators, primarily of caterpillars and beetle larvae [1]. Adult *Podisus* males, and males in some other asopine genera, possess enormous dorsal abdominal glands (DAGs) opening between the third and fourth segments underneath the wings [2, 3]. Homologous sexually dimorphic DAGs have since been reported for two species of phytophagous pentatomoid bugs: the Australian spined citrus bug, *Biprorulus bibax* Breddin (Pentatomidae) [4]; and a West African shield bug, *Sphaerocoris annulus* (F.) (Scutelleridae), whose secretion consists of a mixture of C_9 aliphatic aldehydes [5]. For two North American *Podisus* spp. the male-specific DAG secretions have been chemically elucidated, and shown to function as long-range attractant pheromones [6, 7]. Males of the spined soldier bug, *P. maculiventris* (Say), produce principally (*E*)-2-hexenal, benzyl alcohol and *R*-(+)-α-terpineol, plus lesser amounts of other monoterpenols [6]. In the sympatric species, *P. fretus* Olsen, the male-specific DAG secretion also contains large amounts of (*E*)-2-hexenal and benzyl alcohol, but *S*-(+)-linalool (a minor constituent in the spined soldier bug DAG secretion) is the dominant monoterpenol of *P. fretus* males [7]. Besides attracting potential mates, calling *Podisus* males become vulnerable to a complex of parasitoids that exploit the pheromones as host-finding kairomones, and conspecific males and nymphs are attracted to calling males [8].

We report here on the male DAG chemical compositions of four additional *Podisus* spp.; two Nearctic species (*P. mucronatus* Uhler and *P. placidus* Uhler), and two Neotropical species (*P. connexivus* Bergroth and *P.* sp.).

Materials and Methods

Podisus placidus adults and eggs were collected during the last week in June, 1984, near Gay Head, Martha's Vineyard Island, Massachusetts. Several generations of *P. placidus* were reared in the laboratory on mealworm pupae, *Tenebrio molitor* L.

Reprint requests to Dr. J. R. Aldrich.

Verlag der Zeitschrift für Naturforschung, D-7400 Tübingen
0939–5075/91/0300–0264 $ 01.30/0

(Coleoptera: Tenebrionidae) (Rainbow Mealworms, Compton, CA). Four *P. mucronatus* adult males were collected near Tampa, Florida, on March 27, 1989. The Brazilian *Podisus* spp. were collected in a soybean field near Brasilia, on February 13, 1990. One generation of *P. connexivus* was reared in the laboratory on mealworm pupae; the analysis of *P.* sp. is based on a single field-collected male.

Extracts of the male DAGs were prepared as described in detail previously [6]. In short, the glands were dissected from CO_2-anesthetized bugs submerged in tap water, fat body surrounding the excised glands was removed, and the glands were macerated in 50–200 µl of CH_2Cl_2 or heptane. Part of a male *P. mucronatus* DAG extract (30 µl) was esterified by addition of 10 µl of a diazomethane/ether solution, followed by acetic acid after 2 min. Airborne extracts of some *P. placidus* and *P. connexivus* males were prepared by confining single insects in a 150 ml glass column, drawing air by vacuum (40 ml/min) through *ca.* 30 mg of activated charcoal inside a Sweeny luer-lock filter holder (13 mm; Thomas Scientific, Philadelphia, PA), and extracting the filter with 200 µl of CH_2Cl_2. The contents of the two large nymphal DAGs are shed with each ecdysis making extraction of the exuviae a convenient method for isolation of the combined secretions [2, 3]. Therefore, *P. connexivus* exuviae were collected within a day of ecdysis and extracted with CH_2Cl_2 for analysis.

Samples were analyzed by gas chromatography (GC) on a bonded methyl silicone column (0.25 µm film, 14 m × 0.25 mm ID; DB-1™, J&W Scientific, Folsom, CA) using a Varian 3700 GC with helium as carrier (40 cm/sec), and a temperature program from 45 °C for 2 min to 230 °C at 15 °C/min. One extract from male *P. connexivus* DAGs was analyzed on a Cyclodex-B™ chiral column (0.25 µm film; 30 m × 0.25 mm ID; J&W Scientific) using a Hewlett-Packard 5890 GC with helium as carrier, isothermally at 110 °C. Reported compound percentages are based on mV output from the flame ionization detector using a Shimadzu C-R3A recorder. Electron impact mass spectra (EI-MS) were obtained at 70 eV using a Finnigan 4510 GC-MS, equipped with a 30 m DB-1 column. *Podisus placidus* DAG extracts were also analyzed by chemical ionization mass spectrometry (CI-MS) using NH_3 and ND_3 as reagent gases.

Compounds identified by mass spectral data were cross-checked by GC coinjection of the natural product with an authentic standard under appropriate isothermal conditions. The following standards were obtained commercially: benzyl alcohol, benzaldehyde, linalool, terpinen-4-ol, tiglic acid, tiglyl aldehyde, *n*-tridecane, and 1-nonanol (Aldrich Chemical Co., Milwaukee, WI); (*E*)-2-hexenal, (*E*)-2-hexenol, (*E*)-2-hexenoic acid, (*E*)-2-octenol, (*Z*)-3-nonenol, and benzyl tiglate (Bedoukian Research Inc., Danbury, CN); *trans*-piperitol (PCR Research Chemicals Inc., Gainesville, FL); and α-terpineol (Hercules Inc., Wilmington, DE). (*E*)-2-Hexenyl tiglate, (*E*)-2-hexenyl, (*E*)-2-hexenoate, and (*E*)-2-hexenyl benzoate were synthesized as part of an earlier investigation [9], as were the enantiomers of α-terpineol [6]. (*E*)-4-Oxo-2-hexenal was synthesized according to Ward and VanDorp [10]. 9-Hydroxy-2-nonanone was synthesized from 6-bromo-1-hexanol (from 6-bromohexanoic acid and $BH_3 \cdot THF$) and the sodium salt of ethyl acetoacetate in absolute ethanol by analogy with Vogel [11] (colorless liquid b.p. = 141–164 °C/9 mmHg; 95.3% pure by GC). A standard of 2-(4-hydroxyphenyl)ethanol was prepared by reduction of (4-hydroxyphenyl)acetic acid (Eastman Kodak, Rochester, NY) with $LiAlH_4$ in tetrahydrofuran (pink crystals from water; m.p. = 89–91 °C).

Results

An airborne extract of a single 4-day-old *P. connexivus* male (Fig. 1A) and the extract of the DAGs from the same male (Fig. 1B) exhibited similar patterns by GC, except that the concentration of benzyl alcohol (**2**) is reduced in the aeration sample (2.4% versus 15% in the gland extract). α-Terpineol (**5**) is the major volatile of both the gland extract (72%) and the aeration extract (78%), plus (*E*)-2-hexenal (**1**) (3.2%, DAG extract; 6.0%, airborne extract), linalool (**3**) (2.5%; 3.2%), terpinen-4-ol (**4**) (1.5%; 2.1%), and *trans*-piperitol (**6**) (2.9%; 4.0%) (Fig. 1A and B). The enantiomers of α-terpineol were baseline separated on the Cyclodex-B column; *S*-(−)-α-terpineol eluted at retention time (RT) = 21.5 min and *R*-(+)-α-terpineol eluted at RT = 21.9 min. α-Terpineol from *P. connexivus* consisted of 98% of the *R*-(+)- and 2% of the *S*-(−)-enantiomers. Coinjection of

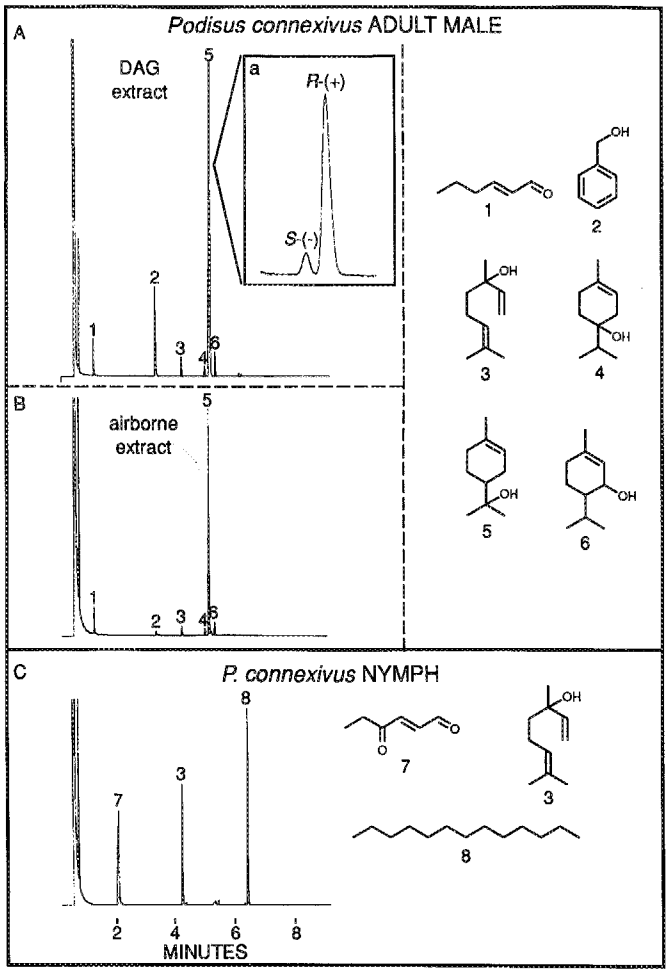

Fig. 1. Gas chromatograms of *Podisus connexivus* exocrine secretions: (A) dorsal abdominal glands (DAGs) from a 5-day-old laboratory-reared adult male, (a) coinjection on a Cyclodex-B chiral column of synthetic *S*-(−)-α-terpineol with male DAG secretion (only RT = 20–24 min shown), (B) airborne extract from the male dissected for (A) aerated for 24 h prior to dissection, and, (C) extract of *ca.* 50 first-fifth instar exuviae (**1** = (*E*)-2-hexenal, **2** = benzyl alcohol, **3** = linalool, **4** = terpinen-4-ol, **5** = α-terpineol, **6** = *trans*-piperitol, **7** = (*E*)-4-oxo-2-hexenal, and **8** = *n*-tridecane).

synthetic *S*-(−)-α-terpineol with the insect-derived material increased the size of the peak at RT = 21.5 min without changing the peak shape (Fig. 1 A, chart speed = 3 cm/min). The exuvial extract of *P. connexivus* is dominated by three compounds: 4-oxo-(*E*)-2-hexenal (**7**) (30%), linalool (**3**) (23%), and *n*-tridecane (**8**) (32%) (Fig. 1 C). The DAG extract of the other Brazilian *Podisus* sp. contained (*E*)-2-hexenal (**1**) (36%), benzyl alcohol (**2**) (14%), and linalool (**3**) (45%) (Fig. 2).

Extracts of male *P. mucronatus* DAGs include a combination of hexenyl, tiglyl, and benzyl derivatives, but lack monoterpenols (Fig. 3). Based upon a pooled extract from 4 males, the principal components are (*E*)-2-hexenal (**1**) (38%), (*E*)-2-hexenol (**10**) (18%), benzyl alcohol (**2**) (13%),

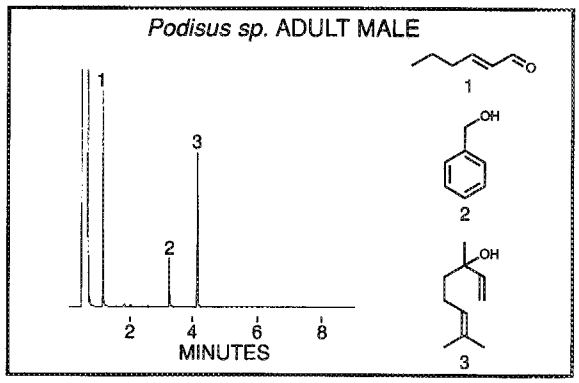

Fig. 2. Gas chromatogram of the dorsal abdominal gland extract from a single field-collected male Brazilian *Podisus* sp. (**1** = (*E*)-2-hexenal, **2** = benzyl alcohol, and **3** = linalool).

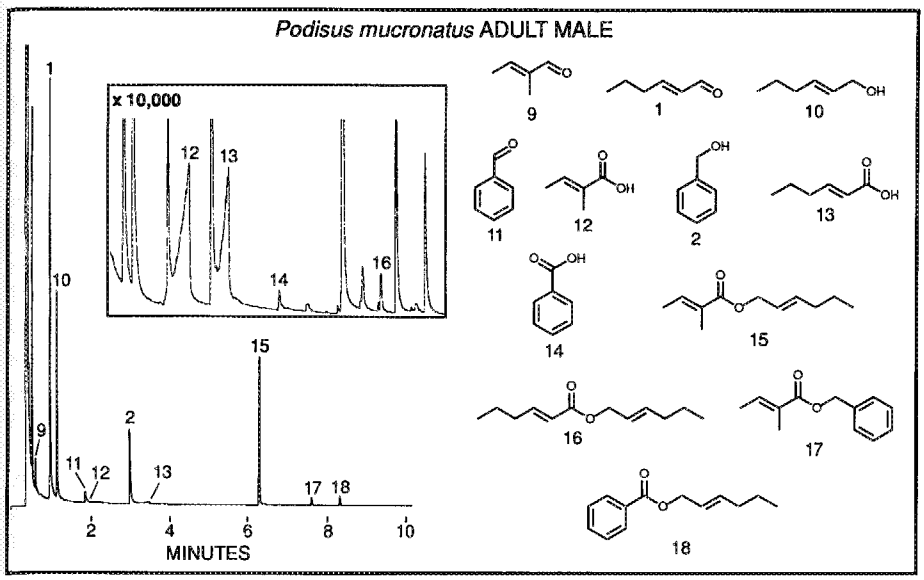

Fig. 3. Gas chromatogram of the dorsal abdominal gland extract from four field-collected *Podisus mucronatus* males (**9** = (*E*)-2-methyl-2-butenal (= tiglyl aldehyde), **1** = (*E*)-2-hexenal, **10** = (*E*)-2-hexenol, **11** = benzaldehyde, **12** = tiglic acid, **2** = benzyl alcohol, **13** = (*E*)-2-hexenoic acid, **14** = benzoic acid, **15** = (*E*)-2-hexenyl tiglate, **16** = (*E*)-2-hexenyl (*E*)-2-hexenoate, **17** = benzyl tiglate, and **18** = (*E*)-2-hexenyl benzoate).

and (*E*)-2-hexenyl tiglate (**15**) (15%). The percentages of (*E*)-2-hexenal and (*E*)-2-hexenol for these males analyzed separately by GC ranged from 40% aldehyde: 18% alcohol to 20% aldehyde: 36% alcohol. In addition, tiglic (**12**), (*E*)-2-hexenoic (**13**), and benzoic (**14**) acids were confirmed by GC-MS of their methyl-ester derivatives. Other identified minor components are tiglyl aldehyde (**9**), benzaldehyde (**11**), (*E*)-2-hexenyl (*E*)-2-hexenoate (**16**), benzyl tiglate (**17**), and (*E*)-2-hexenyl benzoate (**18**).

Gland and aeration extracts of *P. placidus* males also include both (*E*)-2-hexenal and (*E*)-2-hexenol in variable proportions, but the other identified components are heretofore unknown from *Podisus* or other asopines (Fig. 4). The EI-MS of the major component in the DAG extract (Fig. 4A, RT = 6.8 min) suggested 9-hydroxy-2-nonanone as a possible structure. NH_3 CI-MS of this component substantiated this possibility by indicating a molecular weight of 158 (base peak: m/z = 176; $[M + NH_4]^+$), and ND_3 CI-MS confirmed the presence of one exchangeable proton in the molecule (base peak; $m/z = 181$; $[M + ND_4 - H + D]^+$). The EI-MS and RT of synthetic 9-hydroxy-2-nonanone were identical to those for the natural product, verifying the assigned structure (**23**). Other aliphatic components (**19**–**22**) from male *P. placidus* DAG and aeration extracts were identified as for compound **23**. The EI-MS of the minor component eluting just after compound **23** exhibited prominent ions at m/z (%) 77(20), 107(100), and 138(30, M^+), suggesting an hydroxyphenyl ethanol structure for this component [12]. Synthetic 2-(4-hydroxyphenyl)ethanol coeluted with, and gave an identical EI-MS to, the insect-derived material. As for male *P. mucronatus* DAGs, the (*E*)-2-hexenal: (*E*)-2-hexenol ratio in *P. placidus* DAG extracts varied greatly, ranging from 13.5% of the aldehyde with no detectable alcohol to 4.4% aldehyde with 28.1% alcohol. A significant correlation between the age of a male (sampled from < 1-day-old to 29-days-old) and the (*E*)-2-hexenal: (*E*)-2-hexenol ratio was not detected ($r = -0.0596$, $n = 11$). Based upon a pooled sample of DAGs dissected from 90 lab-reared males (Fig. 4A), the *P. placidus* secretion consists of 5.7% (*E*)-2-hexenal (**1**), 20% (*E*)-2-hexenol (**10**), 0.30% (*E*)-2-octenal (**19**), 5.7% (*E*)-2-octenol (**20**), 3.1% (*Z*)-3-nonenol (**21**), 4.2% 1-nonanol (**22**), 57% 9-hydroxy-2-nonanone (**23**), and 0.4% 2-(4-hydroxyphenyl)ethanol (**24**). An airborne extract of

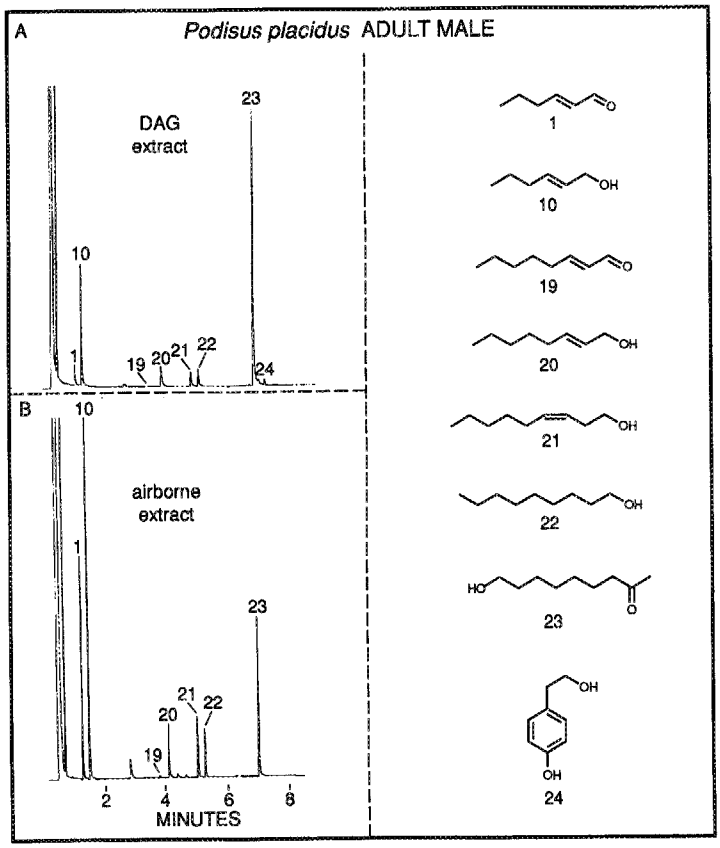

Fig. 4. Gas chromatograms of *Podisus placidus* exocrine secretions: (A) dorsal abdominal gland extract from 90 laboratory-reared adult males and, (B) airborne extract from eight 17–19-day-old laboratory-reared adult males (**1** = (*E*)-2-hexenal, **10** = (*E*)-2-hexenol, **19** = (*E*)-2-octenal, **20** = (*E*)-2-octenol, **21** = (*Z*)-3-nonenol, **22** = 1-nonanol, **23** = 9-hydroxy-2-nonanone, and **24** = 2-(4-hydroxyphenyl)ethanol).

eight 17–29-day-old *P. placidus* males shows the same compounds as identified in the gland extracts (except for compound **24**), but with concentrations increasing with decreasing molecular weights; 17% (*E*)-2-hexenal (**1**), 34% (*E*)-2-hexenol (**10**), 0.37% (*E*)-2-octenal (**19**), 6.4% (*E*)-2-octenol (**20**), 8.1% (*Z*)-3-nonenol (**21**), 5.6% 1-nonanol (**22**), and 21% 9-hydroxy-2-nonanone (**23**) (Fig. 4B).

Discussion

All the compounds identified from the DAG secretions of Brazilian *Podisus* males have been previously identified from North American *Podisus* spp. [2, 3]. In fact, the male DAG secretion of the *Podisus* sp. is quantitatively nearly identical to that for the North American species, *P. fretus* [7], and *P. connexivus* males produce a DAG secretion like that for males of the North American *P. maculiventris* except for a lower proportion of (*E*)-2-hexenal [6]. Furthermore, both *P. connexivus* and *P. maculiventris* [6] males produce predominantly *R*-(+)-α-terpineol in their DAGs. The exocrine blends from nymphs of *P. connexivus* and *P. maculiventris* are alike, as well [3]. While a specific determination was not achieved for the *Podisus* species chemically allied to *P. fretus*, morphologically this Brazilian asopine is clearly distinct from *P. fretus*. Thus, the first two South American *Podisus* spp. to be chemically characterized mirror the two North American species initially investigated. The ranges of the North American species do not overlap the ranges of the South American species [1].

The male DAG chemistry of *P. mucronatus* represents another interesting case of parallel semiochemical evolution among Heteroptera. (*E*)-2-Hexenyl tiglate and (*E*)-2-hexenyl (*E*)-2-hexenoate are the predominant volatiles produced in the male-specific ventral abdominal gland of the Central American bug, *Pachylis laticornis* (Coreidae) [9]. Benzyl tiglate is also a minor secre-

tory constituent of both the pentatomid and the coreid species.

The male DAG secretion of *P. placidus* resembles that of *P. mucronatus* in one respect: (*E*)-2-hexenol is an abundant secretory constituent in both species. Nevertheless, the remaining components identified from the *P. placidus* exocrine blend render the secretion highly distinctive and species-specific. One minor component from the DAG secretion of *P. placidus* provides yet another example of exocrinological parallelism in the Insecta; (*Z*)-3-nonenol is part of the male-produced pheromone of the Mexican fruit fly, *Anastrepha ludens* (Tephritidae) [13].

A pattern now appears to have emerged for *Podisus* attractant pheromones: (*E*)-2-hexenal is omnipresent, but idiosyncratic compounds are biosynthesized in sympatric species.

Acknowledgements

We thank Joseph E. Eger (DowElanco, Tampa, F.L.) for collecting and identifying *Podisus mucronatus*, and Thomas J. Henry (Systematic Entomology Laboratory, USDA-ARS) for identifications of the other North American *Podisus* species. Dr. Jocelia Grazia (Departamento de Zoologia, Instituto de Biociencias, U.F.R.G.S. – Porto Alegre – R.S.) kindly determined the South American species. Dr. Jocelyn Millar (Department of Entomology, University of California, Riverside) performed the chiral analysis, for which we are grateful. Susan Wilzer maintained the *P. placidus* colony and prepared the extract from 90 males. Mention of commercial products does not constitute an endorsement by the U.S. Department of Agriculture.

[1] J. E. McPherson, The Pentatomoidea (Hemiptera) of northeastern North America with emphasis on the fauna of Illinois, Southern Illinois University Press, Carbondale 1982.
[2] J. R. Aldrich, Ann. Rev. Entomol. **33,** 211 (1989).
[3] J. R. Aldrich, in: Biologically active natural products for potential use in agriculture (H. G. Cutler, ed.), American Chemical Society Symposium Series, Washington D.C. 1988.
[4] D. G. James and G. N. Warren, J. Aust. Entomol. Soc. **28,** 75 (1989).
[5] A. J. E. Gough, D. E. Games, B. W. Staddon, D. W. Knight, and T. O. Olagbemiro, Z. Naturforsch. **41C,** 93 (1986).
[6] J. R. Aldrich, J. P. Kochansky, and C. B. Abrams, Environ. Entomol. **13,** 1031 (1984).
[7] J. R. Aldrich, W. R. Lusby, and J. P. Kochansky, Experientia **42,** 583 (1986).
[8] J. R. Aldrich, in: Semiochemistry: Flavors and pheromones (D. M. Soderlund and T. E. Acree, eds.), Walter de Gruyter and Company, Berlin 1985.
[9] J. R. Aldrich, J. P. Kochansky, W. R. Lusby, and S. R. Dutky, J. Chem. Ecol. **8,** 1369 (1982).
[10] J. P. Ward and D. A. VanDorp, Recueil **88,** 989 (1969).
[11] A. I. Vogel, A text-book of practical organic chemistry including qualitative organic analysis, John Wiley & Sons, New York 1962.
[12] P. A. Weibel and M. Hesse, Helv. Chim. Acta **56,** 251 (1973).
[13] D. C. Robacker and W. G. Hart, Entomol. Exp. Appl. **39,** 103 (1985).